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<p>Abundant evidence suggests that overexpression of TGF-<math>\beta</math> by prostate cancer cells enhances their ability to grow and metastasize. TGF-<math>\beta</math> is secreted by cells in a latent form that results from a noncovalent interaction between TGF-<math>\beta</math> and its propeptide (latency-associated peptide, LAP). Mechanisms leading to active TGF-<math>\beta</math> are poorly understood at present. Our lab discovered a mechanism of TGF-<math>\beta</math> activation in which the integrin <math>\alpha</math>V<math>\beta</math>6 binds to an RGD sequence near the C-terminus of LAP. <math>\alpha</math>V<math>\beta</math>6 is only expressed in epithelial cells. We hypothesize that <math>\alpha</math>V<math>\beta</math>6, by activating TGF-<math>\beta</math>1, is an important regulator of normal prostate epithelial proliferation, and that overexpression of <math>\alpha</math>V<math>\beta</math>6 by prostate tumor cells acts in concert with overexpression of its ligand latent TGF-<math>\beta</math>1 to produce active TGF-<math>\beta</math>1 and promote growth of the tumor. In this work, we are testing whether the <math>\beta</math>6 integrin subunit is regulated by androgen, whether it is overexpressed in human prostate cancer, and whether it affects growth and metastasis of prostate cancer in an animal model. Our results to date indicate that <math>\beta</math>6 is expressed in prostate luminal epithelial cells, which activate TGF<math>\beta</math>1 in a <math>\beta</math>6-dependent manner, and that <math>\beta</math>6 expression is upregulated in the mouse in a delayed fashion after castration.</p>			
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## INTRODUCTION

The subject of this work is a system for the activation of latent TGF $\beta$  in the prostate. The system consists of the  $\alpha V\beta 6$  integrin expressed on epithelial cells. Our previous work showed that this integrin can bind to an integrin recognition site (arg-gly-asp) on latent TGF $\beta 1$  and effect its activation [1]. TGF $\beta$  is known to be important for regulating the growth and differentiation of various epithelia, and also to be important in cancer growth. Little is currently known about this system in the prostate: eg, what cells express  $\alpha V\beta 6$ , how expression of the integrin is regulated, and if and when this system regulates prostate epithelial growth via production of active TGF $\beta 1$ . The purpose of the work is to demonstrate whether or not this system plays a role in prostate cancer. The scope of the work involves cell line and mouse experiments (to gauge the normal expression and regulation of  $\alpha V\beta 6$  in the prostate), and evaluation of human prostate cancer tissue and an *in vivo* mouse prostate cancer model (to address the question, does  $\alpha V\beta 6$ -mediated activation of TGF $\beta 1$  promote prostate cancer growth?).

## BODY

The first 12 months of the project addressed tasks 1 (determine  $\beta$ 6 expression in normal prostate and prostate cancer cells) and 2 (determine the effect of androgen on  $\beta$ 6 expression).

We began by looking at well-established prostate cell lines established by Dr. EL Wilson of NYU [2]. One line has characteristics of prostate luminal epithelial (LE) cells, the other of prostate basal epithelial (BE) cells. Because the  $\beta$ 6 integrin is expressed only in epithelial cells, we did not look at other cell types (e.g., prostate smooth muscle cells).

We did immunostaining using a rabbit monoclonal Ab (designated B1) obtained from D Sheppard at UCSF. We found that the LE cells stained intensely for  $\beta$ 6 integrin, whereas the BE cells were negative. We confirmed these results functionally in 2 ways. First, we did adhesion assays using recombinant LAP- $\beta$ 1, the ligand for  $\alpha$ v $\beta$ 6. LE cells, but not BE cells, were able to adhere and spread on plastic coated with LAP- $\beta$ 1 at coating concentrations similar to that needed for other cell types expressing  $\beta$ 6 integrin (e.g., transfected SW-480 colon carcinoma cells). Second, we found that LE cells in culture were able to activate TGF $\beta$ 1 in an  $\alpha$ v $\beta$ 6-dependent manner. This is shown in Figure 1.

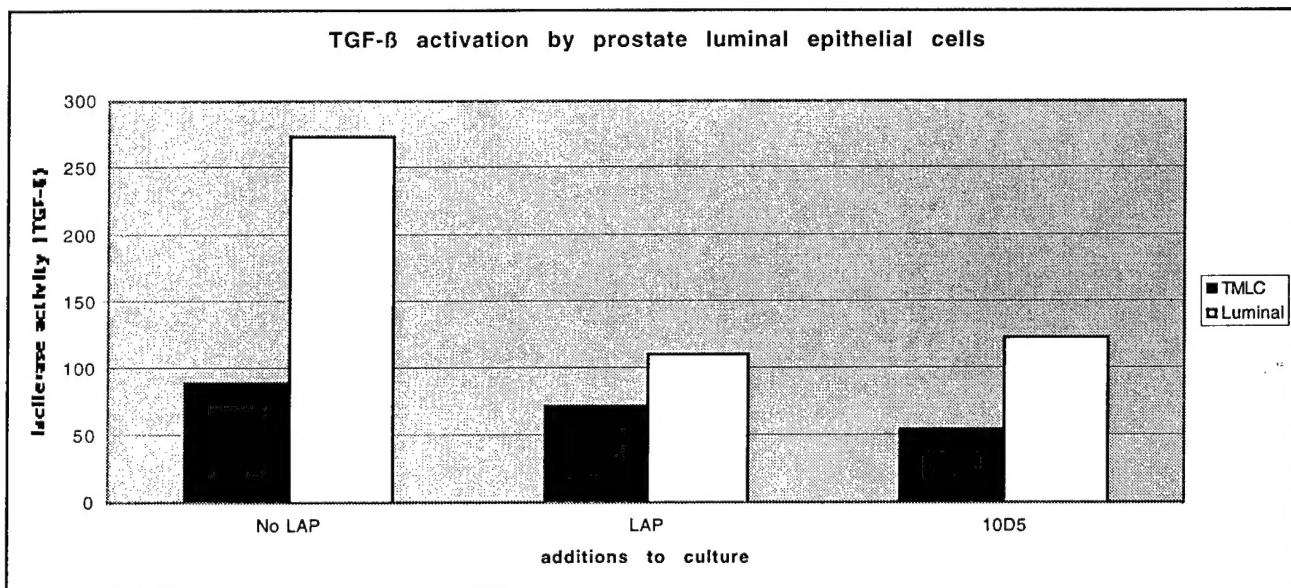


Figure 1. LE cells were cocultured with reporter cells (TMLC) that respond to TGF- $\beta$  by producing luciferase. Compared to reporter cells cultured alone (black bars), reporter cells cultured with LE cells detect active TGF $\beta$  (white bars); the signal is abrogated by a reagent that inhibits active TGF $\beta$  (LAP) and by an anti- $\beta$ 6 Ab (10D5), demonstrating that the active TGF $\beta$  is produced via  $\alpha$ v $\beta$ 6.

We next examined the effect of androgen on  $\beta$ 6 expression by these cells. Both cell types express androgen receptor (AR), and both cell types are normally cultured in the presence of testosterone. Dihydrotestosterone (DHT) is added to the serum-free culture medium of the LE cells, and is present in the 10% serum included in the BE cell medium. Therefore, we set

up subconfluent cultures of LE and BE cells in medium without DHT (for the BE cells this was done by using charcoal-treated serum). After DHT-free growth for 24 or 48 hours, cells were immunostained for  $\beta 6$  integrin. The staining patterns were unchanged: LE cells still expressed a high level of  $\beta 6$  and BE cells were negative. Thus, at least in cell culture, we find no evidence of androgen regulation of  $\beta 6$  expression.

We also examined TGF $\beta$  activation by LE and BE cells in the presence and absence of DHT. As expected, based on the immunostaining results, the BE cells did not activate TGF $\beta$ , regardless of DHT status. However, when LE cells are cultured for 24 h in the absence of DHT and then cocultured with TGF $\beta$ -sensitive reporter cells, we find increased active TGF $\beta$ . This is shown in Figure 2.

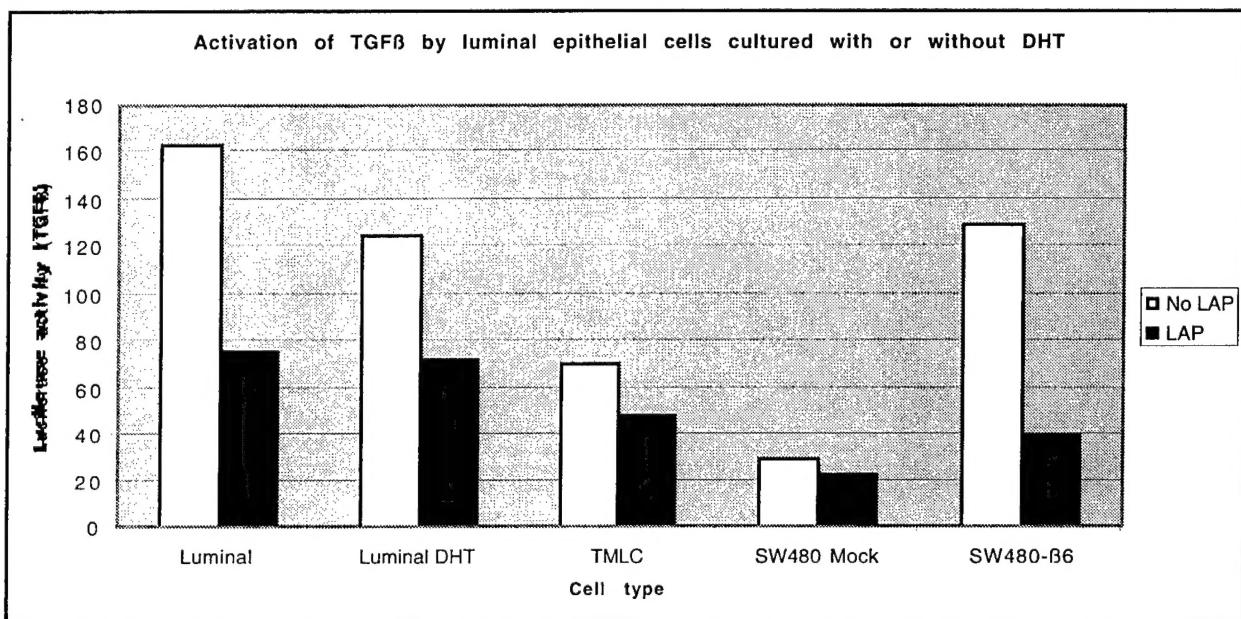


Figure 2. Luminal epithelial cells were cultured with or without DHT for 24 h, then cocultured for an additional 24 hours with TGF $\beta$ -responsive reporter cells to measure production of active TGF $\beta$ . For each condition, cells were cultured with and without a reagent that inhibits active TGF $\beta$  (LAP); in each case, the difference between luciferase activity with and without LAP is proportional to the amount of active TGF $\beta$ . SW480 colon Ca cells mock- or  $\beta 6$ -transfected are shown as negative and positive controls for TGF $\beta$  activation, respectively.

Thus, in short-term cell culture experiments we find that  $\beta 6$  expression is not androgen-regulated, although the  $\beta 6$ -expressing LE cells are able to activate greater amounts of TGF $\beta$  in the absence of DHT. This latter effect might be due to increased TGF $\beta$  expression or to some other effect, such as enhanced interaction of  $\alpha V \beta 6$  with the cytoskeleton.

We have also looked at the regulation of  $\beta 6$  within the murine prostate as a function of androgen. We do this by immunostaining frozen sections of mouse prostate obtained from normal or castrated mice. In normal mouse prostate, we find minimal  $\beta 6$  expression in prostate epithelium. Similarly, in mice that are 2 days and 5 days post-castration we detect only trace amounts of epithelial  $\beta 6$  expression. However, when we

examined prostates from mice 8 weeks post-castration, we found high levels of  $\beta 6$  expression in prostate epithelium.  $\beta 6$  was clearly expressed in luminal cells; in addition, in many areas there was increased staining adjacent to the basement membrane that might represent  $\beta 6$ -expressing basal cells.

Thus, our *in vivo* data suggest that prostate  $\beta 6$  expression is affected by androgen, but in a delayed and therefore perhaps indirect manner. One can speculate that active TGF $\beta$  generated by  $\alpha V \beta 6$  in the involuted prostate contributes to the growth arrest observed in that state.

An obvious test of this idea is to examine  $\beta 6$  knockout mice after castration. This is planned as part of task 3 in the coming year. In anticipation of this, we have acquired the  $\beta 6$  KO mice (on a C57Bl/6 background). Because these mice will also be crossed with a transgenic mouse line (TRAMP mice, which develop prostate cancer), we plan to house the  $\beta 6$  mice in our transgenic facility. Doing so requires that the mice be rederived (superovulated WT females are impregnated by  $\beta 6$  KO males in another facility; the fertilized eggs are removed and implanted in a recipient female within the transgenic facility). Therefore, over the last 4 months we have been working to generate these rederived mice and currently have 5 heterozygous pups and two more pregnant recipient females. Once these mice are available in sufficient numbers, we will examine the histology of  $\beta 6$  KO prostates and compare prostate proliferation (by BrdU labeling) in wild type and  $\beta 6$  KO mice (in the normal state, during prostate involution after castration and then during prostate regeneration following DHT pellet implantation).

In other work, we have made a recent observation that may bear on this work. Work by S Nishimura (UCSF), in which our lab collaborated, has shown that the integrin  $\alpha V \beta 8$  can activate latent TGF $\beta 1$ . This has now been reported in abstract form at the ASCB meeting in December 2000, and a manuscript (on which I am an author) is in preparation.  $\beta 8$  is expressed more widely than is  $\beta 6$  - it is expressed not only in epithelial cells but also in smooth muscle cells and the CNS. There is no published work on  $\beta 8$  expression in the prostate. The importance to this project is that  $\beta 8$  is therefore another potential player as an activator of TGF $\beta$  in the prostate. Currently I do not plan to modify the proposed work to examine  $\beta 8$ ; however, if as we progress observations suggest a major role for  $\beta 8$  in the prostate I may discuss a possible change/addition to our plan with the US Army Medical Research and Materiel Command staff.

## KEY RESEARCH ACCOMPLISHMENTS

- In cell lines,  $\alpha V\beta 6$  is expressed at high levels in luminal but not basal prostate epithelial cells.
- In short-term experiments,  $\beta 6$  is not regulated by androgen in either the murine prostate or in cell lines. However, in long-term experiments,  $\beta 6$  expression is upregulated in the involuted prostate of castrated mice. This is consistent with the idea that  $\beta 6$ -mediated TGF $\beta$  activation is kept at low levels in the normal prostate, but is important in maintaining the involuted state (TGF $\beta$  inhibits epithelial cell growth).
- Although androgen removal does not increase  $\alpha V\beta 6$  expression in luminal prostate cell lines, it does increase TGF $\beta$  activation.

**REPORTABLE OUTCOMES**

None yet. We need data from B6 knockout mice (task 3, year 2) to complement the current results.

## CONCLUSIONS

These experiments are the first to examine the role of  $\alpha V\beta 6$ -mediated activation of latent TGF $\beta$  in the prostate. Our results suggest that normally  $\alpha V\beta 6$  expression in the prostate epithelium is kept at a low level, which (we theorize) is one factor allowing normal expression of the prostate epithelium. Interestingly, there is a delayed expression of  $\beta 6$  integrin after castration, which may contribute to the continued inhibition of epithelial growth in that state.

Our results also show that prostate cell lines (specifically, a luminal cell line) can express  $\alpha V\beta 6$  and activate TGF $\beta 1$  in an  $\alpha V\beta 6$ -dependent manner. Although this particular cell line is not tumorigenic, it is immortalized and p53-null and therefore presumably has some characteristics of prostate cancer cells. Work in the next 2 years will directly examine the expression and influence of  $\alpha V\beta 6$  in malignant prostate cells.

The first year of this project has also been important as a training period in the techniques needed for analyzing murine prostate. We are now in a strong position to extend the work to the subsequent tasks.

"So what." There are many reports in the literature relating cancer outcomes and tumor cell behavior to increased expression of TGF $\beta$  by tumor cells. However, to my knowledge there has never been an analysis of the role of a TGF $\beta$  activator in tumor cells. Yet, our results with  $\alpha V\beta 6$  and lung fibrosis [1] point out the critical role that a TGF $\beta$  activator can play in a TGF $\beta$ -dependent process. If a specific TGF $\beta$  activator can be identified as important in a cancer, this knowledge might be important for determining prognosis and for developing therapies in which the activator is a target.

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